12 Sc

ASSESSMENT OF ACUTE TOXICITY OF HEXACHLOROETHANE IN LABORATORY ANIMALS

STUDY NO. 51-0075-78
JULY - OCTOBER 1976

Approved for public release; distribution unlimited.

US ARMY
ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MD 21010

DEPARTMENT OF THE ARMY
U S. ARMY ENVIRONMENTAL HYGIENE AGENCY Mr. Weeks/cmd/584-3980
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT

9 JAN 1979

SUBJECT: Assessment of Acute Toxicity of Hexachloroethane in Laboratory Animals, Study No. 51-0075-78, July - October 1976

Commander
USA Materiel Development & Readiness Command
ATTN: DRCSG
5001 Eisenhower Avenue
Alexandria, VA 22333

1. This study on acute toxicity of hexachloroethane was performed in support of a request from the US Army Health Clinic, Pine Bluff Arsenal, but the report is such that it could benefit other DOD facilities or activities handling this chemical.

2. A summary of the pertinent findings and recommendations of the inclosed report follows:

The relative toxicity of hexachloroethane was assessed by a review of available data and by experimental studies in animals. Hexachloroethane was found to be moderately toxic orally, produced reversible eye irritation and little or no skin irritation. Hexachloroethane should not pose an acute inhalation hazard except when high vapor concentrations are evolved following contact with hot surfaces. Possible hazards from subchronic and chronic exposures to hexachloroethane were not addressed in this assessment. Development of a firm expression of the overall toxicity of hexachloroethane will depend on the results of studies aimed at subchronic and chronic toxicity in multiple species.

FOR THE COMMANDER:

1 Incl
as (10 cy)

BRENDAN E. JOYCE, Ph.D.
LTC, MSC
Director, Laboratory Services

CF:
Cdr, HSC (HSPA-H)
HQDA (DASG-HCH)
Supt, AHS (HSA-IHE)
Cdr, Pine Bluff Ars (SARPB-MD)
Cdr, MEDDAC, Ft Sill (2 cy)
C, USAEHA-Rgn Div South

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

(14) USAEHA REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: 51-0075-78

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER: (9) Rept.

4. TITLE (and Subtitle): (6) Assessment of Acute Toxicity of Hexachloroethane in Laboratory Animals.

5. TYPE OF REPORT & PERIOD COVERED: Interim Jul — Oct 76

6. PERFORMING ORG. REPORT NUMBER: 51-0075-78

7. AUTHOR(s): (10) MAURICE H. WEEKS, JOSEPH A. THOMASINO, M.D.

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS: US Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD 21010

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 1T1 T Jan 781

11. CONTROLLING OFFICE NAME AND ADDRESS: Commander, US Army Health Services Command, Fort Sam Houston, TX 78234

12. REPORT DATE: (12) Jul - Oct 76

13. NUMBER OF PAGES: 129 25

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report): Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

DDC RECEIVED JAN 23 1978 F

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Hexachloroethane	Dermal LD50 - rabbits
carbon hexachloride	Oral LD50 - rats
perchlorcethane	inhalation toxicology - rats
phenohep	eye irritation - rabbits
1,1,1,2,2,2,Hexachloroethane	skin irritation - rabbits

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The relative toxicity of hexachloroethane was assessed by a review of available data and by experimental studies in animals. Hexachloroethane was found to be moderately toxic orally, produced reversible eye irritation and little or no skin irritation. Hexachloroethane should not pose an acute inhalation hazard except when high vapor concentrations are evolved following contact with hot surfaces. Possible hazards from subchronic and chronic exposures to hexachloroethane were not addressed in this assessment. Development of a firm expression of the overall toxicity of hexachloroethane will depend on the results of studies

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

034 150

Block 20.

aimed at subchronic and chronic toxicity in multiple species.

DEPARTMENT OF THE ARMY
U. S ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT-T/WP

ASSESSMENT OF ACUTE TOXICITY OF HEXACHLOROETHANE*† IN LABORATORY ANIMALS
STUDY NO. 51-0075-78
JULY - OCTOBER 1976

1. AUTHORITY. Letter, SARPB-MD, US Army Health Clinic, Pine Bluff Arsenal, 14 June 1976, subject: Toxicity of Hexachloroethane.

2. REFERENCES.

a. Toxicology Division Procedural Guide, US Army Environmental Hygiene Agency (USAEHA), 1972, revised 1976.

b. Title 29, Code of Federal Regulations (CFR), 1976 ed., Part 1910, Occupational Safety and Health Standards.

c. Title 40, CFR, 1976 ed., Part 162, Regulations for the Enforcement of the Federal Insecticide, Fungicide and Rodenticide Act.

3. PURPOSE. The purpose of this study was to acquire information concerning the toxicity of hexachloroethane by review of available data and by experimental studies in animals. This information provides a basis for advising on possible acute health hazards associated with the handling of this compound in the preparation of smokes, flares, and associated munitions.

---

* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education, and Welfare Publication No. (NIH) 74-23, revised 1972, second printing 1974.
† The experiments reported herein were performed in animal facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

4. BACKGROUND.

a. Hexachloroethane, C_2Cl_6, (carbon hexachloride; perchloroethane; 1,1,1,2,2,2 - hexachloroethane; phenohep; CAS Number 000067721) is a crystalline white solid with a molecular weight of 236.74, bp 186.8°C and density 2.09. It has a camphoraceous odor, readily sublimes without melting and is soluble in alcohol, benzene, chloroform, ether and oil; insoluble in water. It is used as a solvent, in explosives, as a camphor substitute in celluloid, and as a rubber vulcanizing accelerator.[1] It is used in veterinary practice as an anthelminthic for livestock and for treatment of liver fluke in sheep. In a rabbit study using ^{14}C-labelled compound, numerous metabolites are formed following administration, some of which were excreted in the urine and others into the expired air. Only 5 percent of the dose (0.5 g/kg) appeared in the urine, but up to 25 percent of the dose may be eliminated in the expired air. The mechanisms for the formation of the metabolites are not clear, but they probably involve direct removal of chlorine to yield tetrachloroethylene which is a major metabolite of hexachloroethane.[2]

b. The lowest published lethal dosages for hexachloroethane were 325 mg/kg administered intravenously to the dog and 4000 mg/kg given subcutaneously to the rabbit.[3] It has been reported to be moderately irritating to the skin and mucous membranes. Sax gives the material a moderate hazard rating involving both irreversible and reversible changes not severe enough to cause death or permanent injury. He reports that liver injury has been described from exposure to this material.[4]

c. The material is given a slight explosion hazard rating, but is considered a dangerous disaster hazard since, when heated to decomposition, it emits highly toxic fumes of phosgene.[4]

d. The recommended 8-hour Occupational Safety and Health Administration (OSHA) Federal standard for hexachloroethane in the workplace is 1 ppm (9.7 mg/m^3) with a skin notation. Skin refers to the potential contribution to the overall exposure by the cutaneous route including mucous membranes and eyes (reference paragraph 2b).

e. Gleason et al[5] notes the chemical as very toxic causing more potent central nervous effects than chloroform or carbon tetrachloride, but slower in action. On ingestion, severe mucosal injury and often liver necrosis occurs.

f. The OSHA Standard - Air of 1 ppm was recommended because of the serious injury potential to several organ systems of the body. However, because of the paucity of reports of human experience, it is not known whether the 1 ppm limit is sufficiently low to prevent chronic injury in all cases.[b]

g. A literature search using the data base of the National Library of Medicine revealed hexachloroethane had weak therapeutic activity in guinea pigs with experimental opisthorchiasis. It has been used as carriers for evaporation of natural pyrethrins and pyrethroid insecticides.

h. The sample used in these studies was received from Pine Bluff Arsenal, AR, identified as technical grade and manufactured by Hummel Chemical Company, Plainfield, NJ, under Mil H 2358 with National Stock Number (NSN) 6810-00-N00-001.

i. Definitions of selected terms and abbreviations used in this report are found in Appendix A. Numerical data presented in the Appendices are expressed as the mean plus or minus one standard deviation. Statistical significance in this report has been selected at the 0.05 level of probability.

5. FINDINGS. A tabular presentation of animal toxicity data developed in this Agency follows:

TABULAR PRESENTATION OF DATA

Test	Results	Interpretation
SKIN IRRITATION STUDIES		
Rabbits		
Single 24-hour application to intact and abraded skin of New Zealand White rabbits. 0.5 g dry technical grade compound applied to each of six rabbits.	No primary irritation of the intact or abraded skin at 24 or 72 hours or at 7 days. Irritation scores ranged from 0 to 1 with a mode of 0. Results are shown in detail in Appendix D.	Irritation Category IV (reference Appendix B).
0.5 g technical grade compound as a paste in 0.5 ml distilled water applied to each of six rabbits. (Scoring for the evaluation of skin reactions are shown in Appendix C).	No edema and barely perceptible erythema of intact skin areas at 24 hours. Abraded skin areas showed barely perceptible edema formation in one rabbit with moderate to slight erythema reactions. Irritation scores ranged from 0 to 3 with a mode of 0. Results are shown in detail in Appendix E.	Irritation Category III (reference Appendix B). Rubber gloves should be worn when working compound.

Study No. 51-0075-78, Jul - Oct 76

TABULAR PRESENTATION OF DATA

Test	Results	Interpretation
EYE IRRITATION STUDIES		
Rabbits		
Single 24-hour application of 0.1 g dry technical grade compound to one eye of each of six New Zealand White rabbits.	Moderate corneal damage, iritis and conjunctivitis was produced in five of six rabbits at 24, 48 and 72 hour observations. No signs at 7 days. Results are shown in detail in Appendix F.	Irritation Category II (reference Appendix B) Eye protection should be worn when handling this compound. If hexachloroethane should accidentally get into the eyes, it should be immediately washed out with copious amounts of water.

Test	Results	Interpretation
APPROXIMATE LETHAL DOSE (ALD)		
INTRAPERITONEAL INJECTION		
Rats (male) - corn oil diluent	ALD - 2900 mg/kg Toxic signs were ataxia, tremors and convulsions.	Inherent acute toxicity is apparently low.
ORAL ADMINISTRATION		
Rats (male) - corn oil diluent	ALD - 4900 mg/kg Toxic signs were ataxia, red discharge from eyes, tremors and convulsions.	Presents little lethal hazard from acute ingestion.

Study No. 51-0075-78, Jul - Oct 76

TABULAR PRESENTATION OF DATA

Test	Results	Interpretation
LD_{50} STUDIES		
ORAL ADMINISTRATION		
Rats (male) - corn oil diluent	LD_{50} - 5160 mg/kg (95% C.L. 4250-6270 mg/kg) Slope 6.13 SE ± 1.54; Major toxic signs were tremors, ataxia and red discharge around eyes. Results are shown in detail in Appendix G.	Toxicity Category IV (reference Appendix B)
DERMAL APPLICATION		
Rabbits (male) Four rabbits per dosage level - each gram of technical grade material wetted with 1 ml distilled water	LD_{50} ≥32 g/kg No deaths at dosages up to 10 g/kg. Two out of four dead at 32 g/kg. No skin irritation. Results are shown in detail in Appendix H.	Toxicity Category III (reference Appendix B)

Study No. 51-0075-78, Jul - Oct 76

TABULAR PRESENTATION OF DATA

Test	Results	Interpretation
SENSITIZATION STUDIES		
Guinea Pigs (Male)		
Intradermal injection of 0.1 percent suspension (w/v) of hexachloroethane or of a 0.1 percent suspension of dinitro-chlorobenzene (DNCB)* in a mixture containing 1 volume of propylene glycol and 29 volumes of normal saline		
Ten test guinea pigs received and were challenged with a 0.1 percent suspension of hexachloroethane	Challenge dose of hexachloroethane (last intradermal injection after a 2-week rest period) produced no greater response than did the initial injection.	Test compound did not sensitize guinea pigs and is not expected to cause a sensitization reaction in humans.
Ten positive control guinea pigs received and were challenged with a 0.1 percent suspension of DNCB	Positive control (DNCB) produced sensitization in 10 of 10 guinea pigs.	

* A known skin sensitizer

Study No. 51-0075-78, Jul - Oct 76

TABULAR PRESENTATION OF DATA

Test	Results	Interpretation
ACUTE INHALATION VAPOR EXPOSURES		
Single 8-Hour Exposure		
A group of six male rats was exposed to vapors of hexachloroethane at a nominal concentration of 2.5 mg/l (260 ppm). Dispersion tube held at 23°C; chamber flow 1 ℓ/min.	Rats exposed at room temperature to a nominal concentration of 2.5 mg/l for 8 hours showed no toxic signs during exposure or for 14 days thereafter. Body weight gain and organ-to-body weight ratios of the exposed rats were not significantly different from chamber control (reference Appendix J). No exposure-related gross or histopathologic changes were noted in tissues and organs. The following tissue sections were examined from control and exposed rats (260 ppm); eyes, brain, lung, trachea, nasal turbinates, heart, thymus, stomach, small intestine, large intestine, liver, pancreas, spleen, adrenal glands, kidneys, urinary bladder, testes, skin, skeletal muscle, bone and bone marrow.	Compound, at room temperature, should present no acute inhalation hazard from single short-term exposure.

Study No. 5'-0075-78, Jul - Oct 76

TABULAR PRESENTATION OF DATA

Test	Results	Interpretation
A group of six male rats was exposed to vapors of hexachloroethane at a nominal concentration of 57 mg/l (5900 ppm). Dispersion tube held at 50°C; chamber flow at 1 ℓ/min.	Rats exposed to a nominal concentration of 57 mg/l (5900 ppm) for 8 hours showed severe toxic signs including death. At 6 hours, one exposed rat showed a staggered gait, 2 out of 6 were dead at 8 hrs. Surviving rats showed a reduced body weight gain over the 14 days observation period as compared with controls (reference Appendix K). Four exposed (5900 ppm) and four control animals were necropsied 14 days post exposure. No gross exposure-related lesions were observed. Representative tissue sections of the following organs and tissues were examined histologically; eyes, brain, lung, trachea, nasal turbinates, heart, thymus, stomach, small intestine, large intestine, liver, pancreas, spleen, adrenal glands, kidneys, urinary bladder, testes, skin, skeletal muscle, bone and bone marrow. Exposure- related lesions were observed in two of the four exposed animals. The lesions in both animals were a subacute diffuse interstial pneumonitis of minimal to moderate severity. Vascular congestion in association with these lung changes were also observed. A purulent exudate was observed in the nasal turbinates from one of four control and one of four exposed animals. This change was not deemed exposure-related, but an indication of a low-grade edemic upper respiratory disease.	Compound is moderately toxic at high concentration and exposure should be avoided. Compound should not be handled at elevated temperatures without respiratory protection or in hoods.
A group of six male rats were exposed to room air only and served as chamber controls.		

Study No. 51-0075-78, Jul - Oct 76

TABULAR PRESENTATION OF DATA

Test	Results	Interpretation
Single 6-Hour Exposure		
A group of six male rats was exposed to vapors of hexachloroethane at a nominal concentration of 17 mg/l (1000 ppm). Dispersion tube held at 50°C; chamber flow at 1 ℓ/min.	Rats exposed to a nominal concentration of 17 mg/l (1000 ppm) for 6 hours showed staggered gait (2 out of 5) and reduced weight gain after 24 hours followed by normal rate up to 14 days (reference Appendix L). No exposure-related gross or histopathologic changes were noted in tissues and organs.	Handling hexachloroethane at elevated temperatures should be avoided unless wearing respiratory protection or in hoods.
A group of six male rats were exposed to room air only and served as chamber controls.	The following tissue sections were examined from control and exposed (1000 ppm) rats: eyes, brain, lung, trachea, nasal turbinates, heart, thymus, stomach, small intestine, large intestine, liver, pancreas, spleen, adrenal glands, kidneys, urinary bladder, testes, skin, skeletal muscle, bone and bone marrow.	

Study No. 51-0075-78, Jul - Oct 76

TABULAR PRESENTATION OF DATA

Test	Results	Interpretation
MUTAGENICITY PLATE ASSAY† (In Vitro Mutagenic Evaluation)		
A study was performed to evaluate hexachloroethane for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparation.	Nonactivation Tests Tests conducted on hexachloroethane in the absence of a metabolic system were all negative.	Hexachloroethane did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and is considered not mutagenic under these test conditions.
One yeast strain, Saccharomyces cerevisiae (D4), and five bacteria strains of Salmonella typhimurium (TA-1535, TA-1537, TA-1538, TA-98, TA-100) were used in evaluating mutagenic potential. The compound was tested directly and in the presence of liver microsomal enzyme preparations from rats pretreated with Aroclor®. The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The doses employed for the evaluation of hexachloroethane were 0.1, 1.0, 10, 100 and 500 µg of this compound per plate. The solvent used was Dimethyl Sulfoxide.	Activation Tests Tests conducted on hexachloroethane in the presence of the rat liver activation system were all negative.	

† Work performed under contract by Litton Bionetics, Inc., Kensington, MD (LBI Project No. 2683, 24 November 1976).
® Aroclor is a registered trademark of Monsanto Chemical Co., 800 N. Lindberg Blvd, St Louis, MO. Use of trade names does not imply endorsement by the US Army, but is used only in identification of a specific product.

6. DISCUSSION. Previously reported studies indicated that this compound posed a hazard from skin and inhalation exposures. Results from acute toxicity studies reported here do not support those assumptions, i.e., toxic signs from single inhalation exposures only at 1000 ppm and a dermal LD_{50} equal to or greater than 32 g/kg. It was also only moderately toxic orally, produced reversible eye irritation and little or no skin irritation. Although it sublimes at room temperature, hexachloroethane does not seem to pose an acute inhalation hazard except perhaps from circumstances where the compound would accidentally come into contact with hot surfaces. The subject of species differences and subchronic and chronic exposures needs to be addressed before firm conclusions as to its overall toxicity can be considered.

7. RECOMMENDATION. It is recommended that long-term and multiple species exposures be addressed in order to more fully develop a firm expression of the inherent toxicity of hexachloroethane.

MAURICE H. WEEKS
Chief, Toxicity Evaluation Branch
Toxicology Division

JOSEPH A. THOMASINO, M.D.
CPT, MC
Occupational Medicine Officer
Occupational and Environmental
Medicine Division

APPROVED:

ARTHUR McCREESH, Ph.D.
Chief, Toxicology Division

ROBERT W. FELLINI, M.D.
LTC, MC
Chief, Occupational and Environmental
Medicine Division

APPENDIX A

GLOSSARY OF RECURRING DEFINITIONS, ABBREVIATIONS AND SYMBOLS USED BY THE TOXICOLOGY DIVISION, USAEHA

Definitions of medical terms and abbreviations used in this report are in agreement with Stedman's Medical Dictionary, 20th Edition, published by the Williams and Wilkins Company, Baltimore, MD (1961). The following terms and abbreviations are either not found in the above reference or have been modified to fit the special purposes of this report. Some of the terms have been included below for special emphasis.

DEFINITIONS

WORD	DEFINITION
Acute Exposure	One exposure to exogenous test material for no longer than 8 hours. Animals are normally observed for 14 days after exposure.
Approximate Lethal Dose	In range finding the first dose of the lowest series of three ascending doses (each being 50 percent higher in concentration than the previous) all of which produce fatalities.
Hazard Evaluation	A study performed to estimate the degree of danger associated with the use of a material under specified conditions of use.
Nominal Concentration	Concentration of compound in the exposure chambers as determined by ascertaining the weight of the sample lost from the dispersion apparatus divided by total volume of chamber air used throughout the exposure time.
Primary Irritation	A local inflammatory reaction of the skin, produced by a compound, which does not produce destruction or irreversible change at the site of contact.
Subchronic Exposure	Repeated daily or constant exposure to a test material for no longer than 179 days nor less than 2 days. Post observation period will vary.

Study No. 51-0075-78, Jul - Oct 76

Technical Grade Compound	As produced by the manufacturers of their commercial compound; definition dependent upon manufacturer's criteria.

Symbol	Meaning
>	is greater than
<	is less than
ℓ/min	liters per minute
mg/l	milligrams of compound per liter of air
g	gram
≥	equal to or greater than

APPENDIX B

TOXICITY CATEGORIES: 40 CFR 162

Hazard Indicators	I	II	III	IV
Oral LD_{50}	Up to and including 50 mg/kg	From 50 through 500 mg/kg	From 500 through 5,000 mg/kg	Greater than 5,000 mg/kg
Inhalation LC_{50}:				
(a) Dust or mist	Up to and including 2.0 mg/l	From 2.0 through 20	From 20 through 200	Greater than 200
(b) Gas or vapor	Up to and including 200 p/m	From 200 through 2,000	From 2,000 through 20,000	Greater than 20,000
Dermal LD_{50}	Up to and including 200 mg/kg	From 200 through 2,000	From 2,000 through 20,000	Greater than 20,000
Eye effects	Irreversible corneal opacity at 7 days	Corneal opacity reversible within 7 days or irritation persisting for 7 days	No corneal opacity irritation reversible within 7 days	No irritation
Skin irritation	Severe irritation or damage at 72 hours	Moderate irritation at 72 hours	Mild or slight irritation at 72 hours	No irritation at 72 hours

APPENDIX C

EVALUATION OF SKIN REACTIONS*

Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beet redness to slight eschar formation)	4

Edema Formation

No edema	0
Very slight (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (edges raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extenoing beyond area of exposure)	4

* An individual irritation score is equal to the sum of the scores for edema formation and erythema and eschar formation.

Study No. 51-0075-78, Jul - Oct 76

APPENDIX D

COMPOUND: Hexachloroethane

PRIMARY SKIN EFFECTS NEW ZEALAND WHITE RABBITS	TOXICITY CATEGORY * IV	CONDITIONS - Single 24-hour application of 0.5 g dry white crystalline compound per skin application site

	Time of Observation	Response Rabbit No.							
	Hours	1	2	3	4	5	6	Mean Score	Comments
Erythema & Eschar									
Intact Skin	24	1		1		0		0.67	Cat IV compounds are compounds producing no primary irritation of the intact skin and the skin surrounding an abrasion.
Intact Skin	72	0		1		0		0.33	
Abraded Skin	24		0		0		0	0	
Abraded Skin	72		0		0		0	0	
						Subtotal		1.0	
Edema Formation									
Intact Skin	24	0		0		0		0	
Intact Skin	72	0		0		0		0	
Abraded Skin	24		0		0		0	0	
Abraded Skin	72		0		0		0	0	
						Subtotal		0	
						Total		1.0	

* 40 CFR 162

Study No. 51-0075-78, Jul - Oct 76

APPENDIX E

COMPOUND: Hexachloroethane

PRIMARY SKIN EFFECTS NEW ZEALAND WHITE RABBITS	TOXICITY CATEGORY* III	CONDITIONS - 0.5 gram moistened compound/ application site. Each gram of crystalline compound moistened with 1 ml distilled water

	Time of Observation	Response Rabbit No.							
	Hours	1	2	3	4	5	6	Mean Score	Comments
Erythema & Eschar									
Intact Skin	24			1		1	1	1.0	Cat III compounds are compounds producing mild primary irritation of the intact skin and of the skin surrounding an abrasion.
Intact Skin	72			0		0	1	0.33	
Abraded Skin	24	3	1		1			1.67	
Abraded Skin	72	2	0		0			0.67	
						Subtotal		3.67	
Edema Formation									
Intact Skin	24			0		0	0	0	
Intact Skin	72			0		0	0	0	
Abraded Skin	24	1	0		0			0.33	
Abraded Skin	72	0	0		0			0	
						Subtotal		0.33	
						Total		4.00	

* 40 CFR 162

Study No. 51-0075-78, Jul - Oct 76

APPENDIX F

COMPOUND: Hexachloroethane									
ACUTE EYE EFFECTS NEW ZEALAND WHITE RABBITS		TOXICITY CATEGORY* II						CONDITIONS - Single 24-hour application of 0.1 g of dry white crystalline compound to one eye of each rabbit	
Time of Reading Hrs-Days	Structure	Scores Rabbit No. 1	2	3	4	5	6	Mean Score	Comments
24	Cornea	2	0	2	2	2	1	1.5	Cat II compounds are compounds producing mild injury to the cornea and, in addition, some injury to conjunctiva with corneal opacity reversible within 7 days.
	Iris	1	0	1	1	1	1	0.8	
	Conjunctivae	7	4	4	6	6	6	5.5	
48	Cornea	1	1	1	0	1	1-	0.8	
	Iris	0	0	0	0	0	0	0.0	
	Conjunctivae	4	4	4	4	5	3	4.0	
72	Cornea	0	0	1	0	0	0	0.2	
	Iris	0	0	0	0	0	0	0.0	
	Conjunctivae	1	1	2	1	0	1	1.0	
7-Days	Cornea	0	0	0	0	0	0	0.0	
	Iris	0	0	0	0	0	0	0.0	
	Conjunctivae	0	0	0	0	0	0	0.0	

* 40 CFR 162

Study No. 51-0075-78, Jul - Oct 76

APPENDIX G

COMPOUND: Hexachloroethane — TOXICITY CATEGORY* IV

ACUTE ORAL LD_{50} MALE RATS SPRAGUE-DAWLEY, WISTAR

LD_{50}* 5160 mg/kg — 95% C.L. 4250-6270 mg/kg

Slope 6.13 — S.E. 1.54

Conditions Administered as 50% solution in corn oil (Mazola)

Dosage	Conc %	Onset of signs (s), mortality (m) Hours 0-4	Hours 4-12	Hours 12-24	Days 2	3	4	5	6	7	8-14	Mort Cumulative	Mean Body Wt. Init	Mean Body Wt. Fin	Mean Body Wts. (g) Days 3	7	14
2510	50		S									0/6	272 ±4	272 ±16	228 ±9	253 ±12	272 ±16
3160	50		S		M	M						2/6	210 ±9	272 ±10	221 ±12	249 ±10	272 ±10
3980	50		S									0/6	205 ±6	251 ±30	192 ±41	221 ±29	251 ±30
5010	50		S	M2								2/6	210 ±7	254 ±18	207 ±11	227 ±13	254 ±18
6310	50		S	M3	M					M		5/6	210 ±4	250	187	217	250
7940	50		S	M	M3	M						5/6	210 ±4	215	164	190	215
10000	50		S	M	M2	M2	M					6/6	205 ±6	-	-	-	-

Signs of Intoxication: Red exudate appeared around eyes day of dosing at all dosages and persisted throughout 14-day observation period. Tremors, ataxia, gasping appeared in dosages of 5010 mg/kg and higher and persisted for 3 days.

Gross Autopsy: Decedents: One rat showed degeneration of the kidney cortex.

Survivors: No gross lesions except those attributable to euthanasia.

* 40 CFR 162

APPENDIX H

COMPOUND: Hexachloroethane — TOXICITY CATEGORY* III

ACUTE DERMAL LD_{50}
MALE RABBITS
NEW ZEALAND WHITE

LD_{50}* ~ 32 g/kg — 95% C.L. Not calculated

Slope Not calculated — S.E. Not calculated

Condition: Each gram of applied grandular compound moistened with 1 ml distilled water

Dosage g/kg	Conc %	Onset of signs (s), mortality (m) Hours 0-4	Hours 4-12	Hours 12-24	Days 2	Days 3	Days 4	Days 5	Days 6	Days 7	Days 8-14	Mort Cumulative	Mean Body Wt. Init kg	Mean Body Wt. Fin kg	Mean Body Wts. (kg) Days 1	Days 6	Days 14
1.0												0/4	3.66 ±.56	2.74 ±.35	3.30 ±.50	2.78 ±.13	2.74 ±.35
3.2												0/4	2.80 ±.38	3.41 ±.50	2.63 ±.45	3.19 ±.49	3.41 ±.50
10.0												0/4	2.41 ±.14	3.09 ±.67	2.28 ±.19	2.97 ±.68	3.09 ±.67
32.0							M2					2/4	3.46 ±.39	2.18 ±1.10	2.13 ±.46	2.46 ±.33	2.18 ±1.10
Control (0)												0/4	3.46 ±.39	3.19 ±.59	3.15 ±.39	3.21 ±.63	3.19 ±.59

Signs of Intoxication: Body weight loss

Dermal Irritation: No irritation

Gross Autopsy: Decedents: No gross lesions

Survivors: Of the 18 rabbits, two showed congestion of the kidney cortex and one showed hyperemia of the gastric mucosa at 32 mg/kg

* 40 CFR 162

Study No. 51-0075-78, Jul - Oct 76

APPENDIX I

COMPOUND: Hexachloroethane

GUINEA PIG SENSITIZATION
MALE
HARTLEY STRAIN

Substance: Hexachloroethane

Identity: Intradermal injection - ten sensitizing doses of 0.1 ml of a 0.1 percent solution in saline

Positive Control: Dinitrochlorobenzene (DNCB)

	Mean Body Weight (g)		Mean Irritation Scores				Comments
			Diluent		Test Compound		
24 Hrs	Initial	Final	Initial	Final	Initial	Final	
Test Cmpd	258 ±27	450 ±28	0	0	0.8	0.4	Test compound did not produce a sensitization reaction in guinea pigs.
Positive Control	294 ±31	494 ±45	0	0	19 ±11	341 ±85	DNCB positive control showed a sensitizing reaction in 10/10 guinea pigs.
	Mean Body Weight (g)		Diluent		Test Compound		
48 Hrs	Initial	Final	Initial	Final	Initial	Final	
Test Cmpd	-	-	0	0	0	0.2	Final Scores
Positive Control			0	0	5 -3	230 ±88	>100 - Strong Sensitizing 25-100 - Mild Sensitizing <25 - No Sensitizing

Study No. 51-0075-78, Jul - Oct 76

APPENDIX J

ACUTE INHALATION EXPOSURE
SINGLE 8-HOUR EXPOSURE OF MALE RATS
HEXACHLOROETHANE VAPOR

TABLE 1. MEAN BODY WEIGHT (g)

	Preexposure	Post Exposure			
	Day	Day			
Treatment Group	0	1	3	7	14
Chamber Control	122 ±5	130 ±6	153 ±6	178 ±8	227 ±14
Exposure at 23°C	124 ±8	124 ±8	143 ±11	172 ±16	216 ±21

TABLE 2. ORGAN-TO-BODY WEIGHT RATIOS OF MALE RATS NECROPSIED 14 DAYS AFTER EXPOSURE

Treatment Group	Mean Terminal Body Weight (g)	Mean Organ-to-Body Weight Ratios Grams per 100 Grams Body Weight Liver	Kidney	Spleen	Lung	Testes
Chamber Control	227 ±14	4.7 ±0.2	0.9 ±0.1	0.42 ±0.04	0.7 ±0.1	1.1 ±0.1
Exposure at 23°C	216 ±21	5.1 ±0.3	0.9 ±0.1	0.35 ±0.05	0.6 ±0.1	1.1 ±0.2

Study No. 51-0075-78, Jul - Oct 76

APPENDIX K

ACUTE INHALATION EXPOSURE
SINGLE 8-HOUR EXPOSURE OF MALE RATS
HEXACHLOROETHANE VAPOR

TABLE 1. MEAN BODY WEIGHT (g)

	Preexposure	Post Exposure			
	Day	Day			
Treatment Group	0	1	3	7	14
Chamber Control	125 ±6	128 ±6	144 ±7	171 ±10	220 ±13
Exposure at 50°C	116* ±12	101* ±13	117* ±14	145* ±10	188 ±18

* Significantly different from controls at p 0.01 level of probability.

TABLE 2. ORGAN-TO-BODY WEIGHT RATIOS OF MALE RATS NECROPSIED 14 DAYS AFTER EXPOSURE

Treatment Group	Mean Terminal Body Weight (g)	Mean Organ-to-Body Weight Ratios Grams per 100 Grams Body Weight				
		Liver	Kidney	Spleen	Lung	Testes
Chamber Control	220 ±13	4.5 ±0.2	0.9 ±0.1	0.4 ±0.1	0.7 ±0.1	1.1 ±0.1
Exposure at 50°C	188 ±18	4.3 ±0.2	0.8 ±0.1	0.4 ±0.1	0.7 ±0.1	1.1 ±0.1

Study No. 51-0075-78, Jul - Oct 76

APPENDIX L

ACUTE INHALATION EXPOSURE
SINGLE 6-HOUR EXPOSURE OF MALE RATS
HEXACHLOROETHANE VAPOR

TABLE 1. MEAN BODY WEIGHT (g)

	Preexposure Day	Post Exposure Day			
Treatment Group	0	1	3	7	14
Chamber Control	110 ±10	112 ±10	133 ±9	146 ±12	192 ±11
Exposure at 50°C	113 ±7	106 ±3	128 ±6	153 ±7	200 ±10

TABLE 2. ORGAN-TO-BODY WEIGHT RATIOS OF MALE RATS NECROPSIED 14 DAYS AFTER EXPOSURE

Treatment Group	Mean Terminal Body Weight (g)	Mean Organ-to-Body Weight Ratios Grams per 100 Grams Body Weight Liver	Kidney	Spleen	Lung	Testes
Chamber Control	192 ±11	5.0 ±0.3	0.9 ±0.1	0.4 ±0.1	0.7 ±0.1	1.2 ±0.1
Exposure at 50°C	200 ±10	5.2 ±0.1	0.9 ±0.1	0.4 ±0.1	0.7 ±0.1	1.1 ±0.2

APPENDIX M

BIBLIOGRAPHY

1. The Merck Index, 9th ed., Merck and Company, Inc., Rahway, NJ, p 612 (1976)

2. W. R. Jondorf, D. V. Parke and R. T. Williams, Biochem J 65:14 ff (1957); cited by R. T. Williams in Detoxification Mechanisms, J. Wiley, New York, NY, p 30 (1959)

3. G. S. Barsoum and K. Saad, Relative Toxicity of Certain Chlorine Derivatives of the Aliphatic Series, Quart J Pharm and Pharmacol, 7, p 205 (1934)

4. N. Irving Sax, Dangerous Properties of Industrial Materials, 4th ed., Van Nostrand Reinhold Company, New York, NY, p 804 (1975)

5. Marion N. Gleason, Robert E. Gosselin, Harold C. Hodge and Roger P. Smith, Clinical Toxicology of Commercial Products, 4th ed., The Williams and Wilkins Company, Baltimore, MD, p 112 (1976)

6. Documentation of the Threshold Limit Values, American Conference of Governmental Industrial Hygienists, 3d ed., p 124 (1971)